FBI pH 5.5	Clone	Km	Vmax	Vmax/Km	X improved
50-225 uM [S]	4F13 G12	62	15	0.242	7.1
	4F15 A11	58	13.6	0.234	6.9
	4F15 C3	39	9	0.231	6.8
	4F6 A11	191	28.3	0.148	4.4
	4F3 B5	101.8	16.4	0.161	4.7
	4F2 G10	41.2	9.4	0.228	6.7
	4F19 F2	235	59.5	0.253	7.4
	4F21 C8	113	22	0.195	5.7
	4F22 B2	161	21.5	0.134	3.9
	4F28 G1	172	23.9	0.139	4.1
	WT	349	11.8	0.034	1

22 hits (>3X Improved)

AP1 pH 5.5	Clone	Km	Vmax	Vmax/Km	X improved
50-225 uM [S]	4F13 G12	365	44.2	0.121	6.1
	4F15 A11	438	46	0.105	5.3
	4F15 C3	563	27.6	0.049	2.5
	4F6 A11	288	28.3	0.098	4.9
	4F3 B5	378	25.3	0.067	3.4
	4F2 G10	2209	61.3	0.028	1.4
	4F19 F2	652	18	0.028	1.4
	4F21 C8	305.5	55.9	0.183	9.2
	4F22 B2	444	64.5	0.145	7.3
	4F28 G1	ND	ND	ND	ND
	WT	450	9.2	0.02	1

11 hits (>3X Improved)

Fig. 1

Results: Kinetic parameters of pH-optimized candidates
H1 and B12 compared to wild type APAO

	FB1, pH 5.5				*AP1, pH 5.5*			
Vari-ant	*kcat*	*km*	*kcat /km*	*Fold Impr*	*kcat*	*km*	*kcat /km*	*Fold Impr*
WT	150.0	98.0	1.5	1.0	240.0	430.0	0.6	1.0
H1	2800.0	70.0	40.0	26.1	662.0	544.0	1.2	2.2
B12	701.0	62.0	11.3	7.4	200.0	280.0	0.7	1.3

Fig. 2

H1 and B12 share eight amino acid changes from wild type.

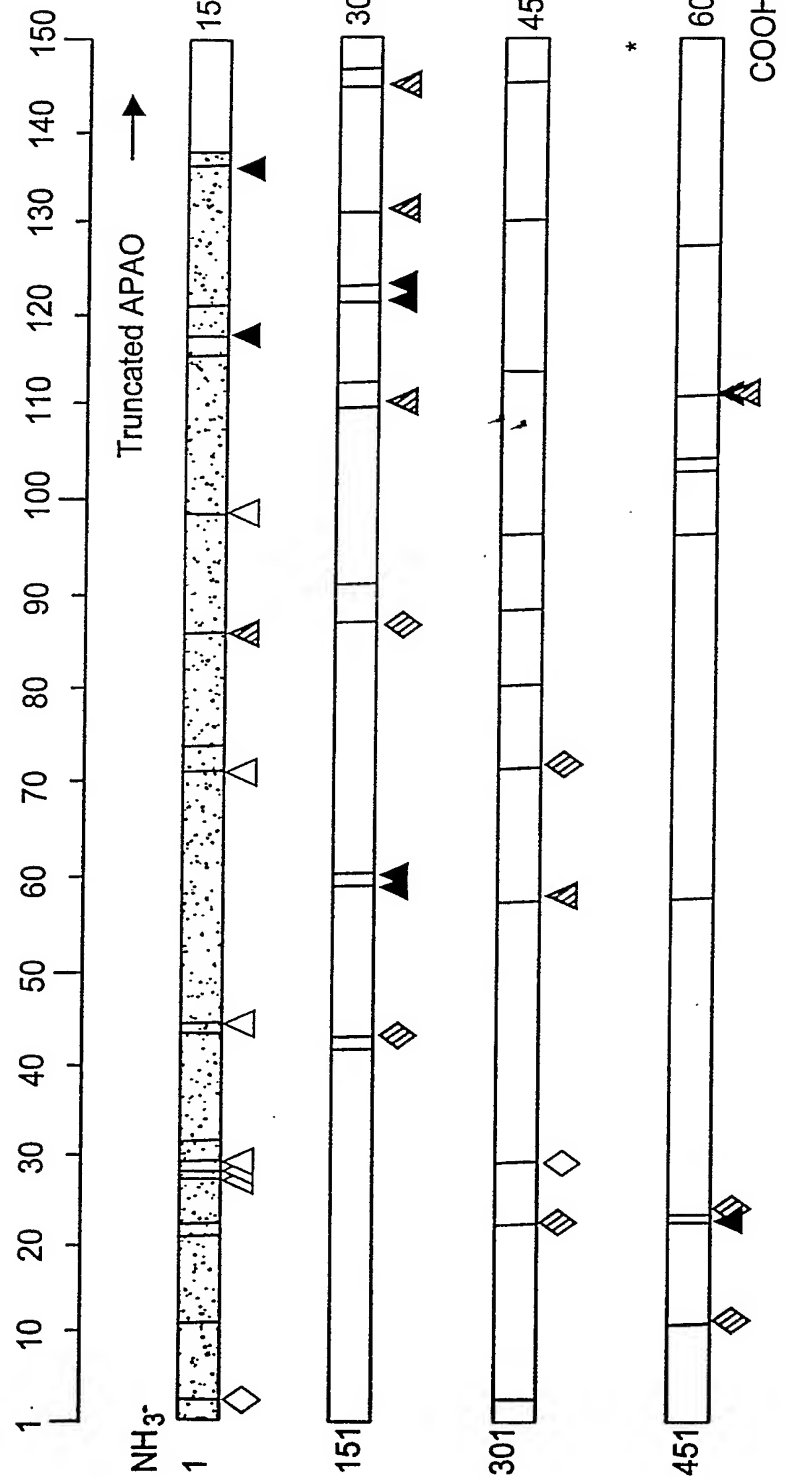
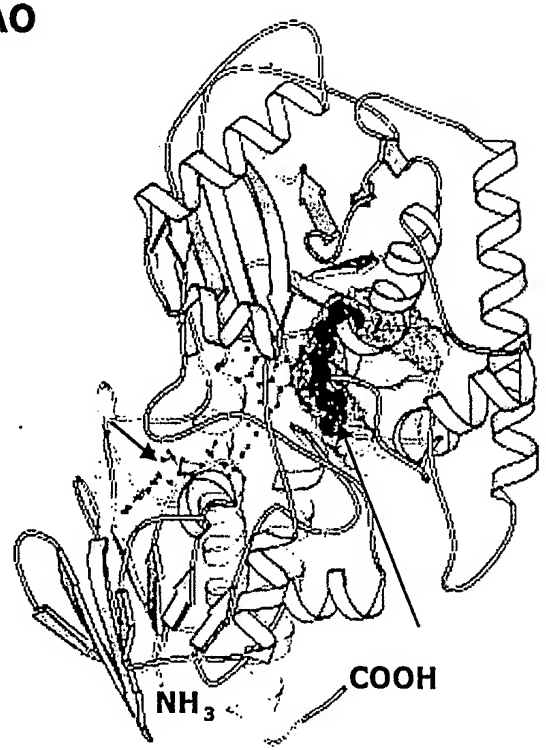


Fig. 3

Several H1 & B12 mutations map to a putative substrate binding region of APAO

MPAO



Maize Polyamine Oxidase (MPAO) 30 Å crystal structure (Binda *et al.* 1999. Structure 7:265) Substrate "tunnel" shown in wire form.

APAO

APAO (truncated, amino acids 142 -600) 3-D Model after Binda *et al.* Putative substrate "tunnel" shown in center right.

Mutations: B12 △◇ H1 ◭◈ Both ▲♦

Fig. 4

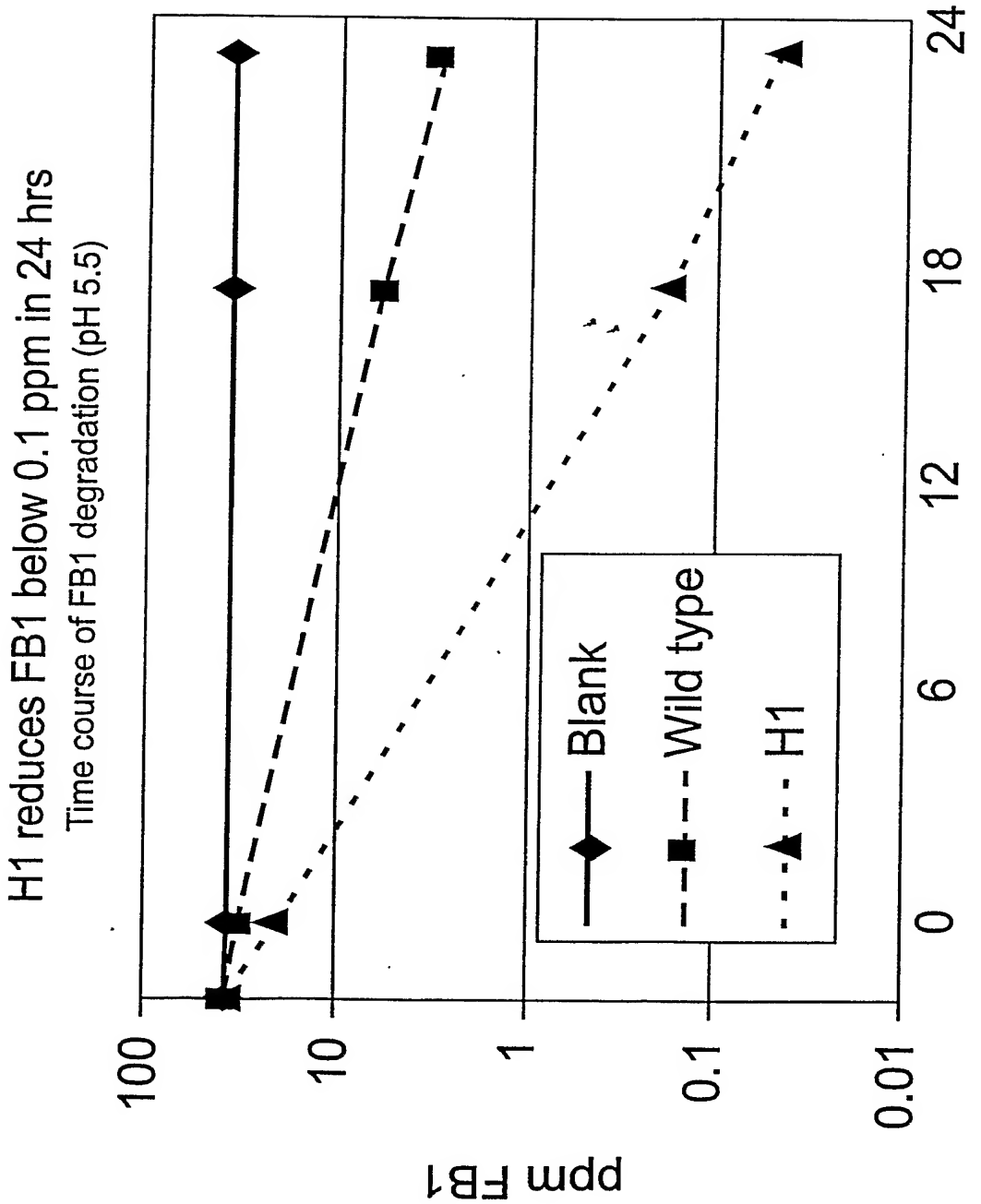

H1 reduces FB1 below 0.1 ppm in 24 hrs
Time course of FB1 degradation (pH 5.5)
ppm FB1
100
10
1
0.1
0.01
0
6
12
18
24
Time (Hours)
Blank
Wild type
H1

Fig. 5

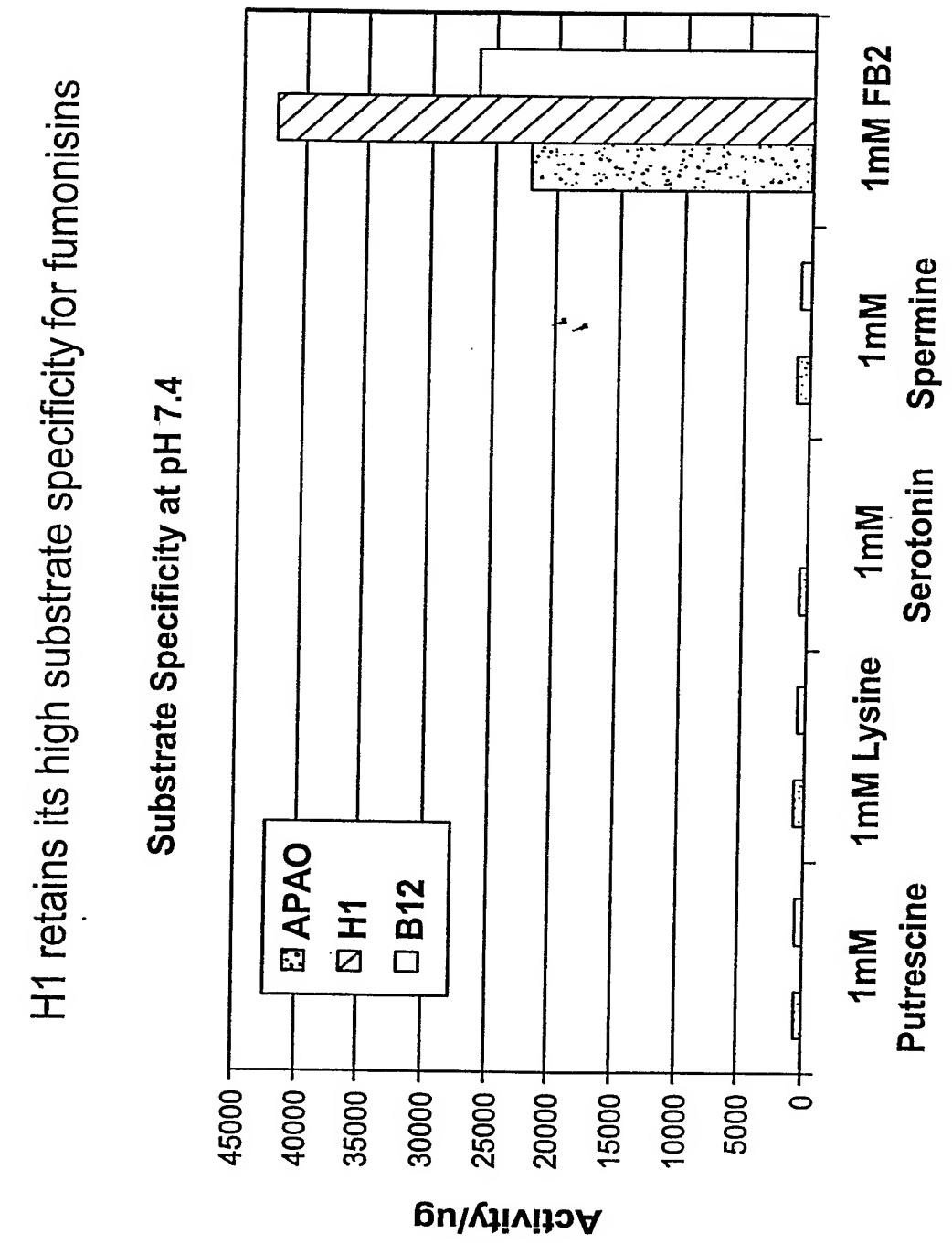

H1 retains its high substrate specificity for fumonisins
Substrate Specificity at pH 7.4
APAO
H1
B12
Activity/ug
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
1mM Putrescine
1mM Lysine
1mM Serotonin
1mM Spermine
1mM FB2

Fig. 6

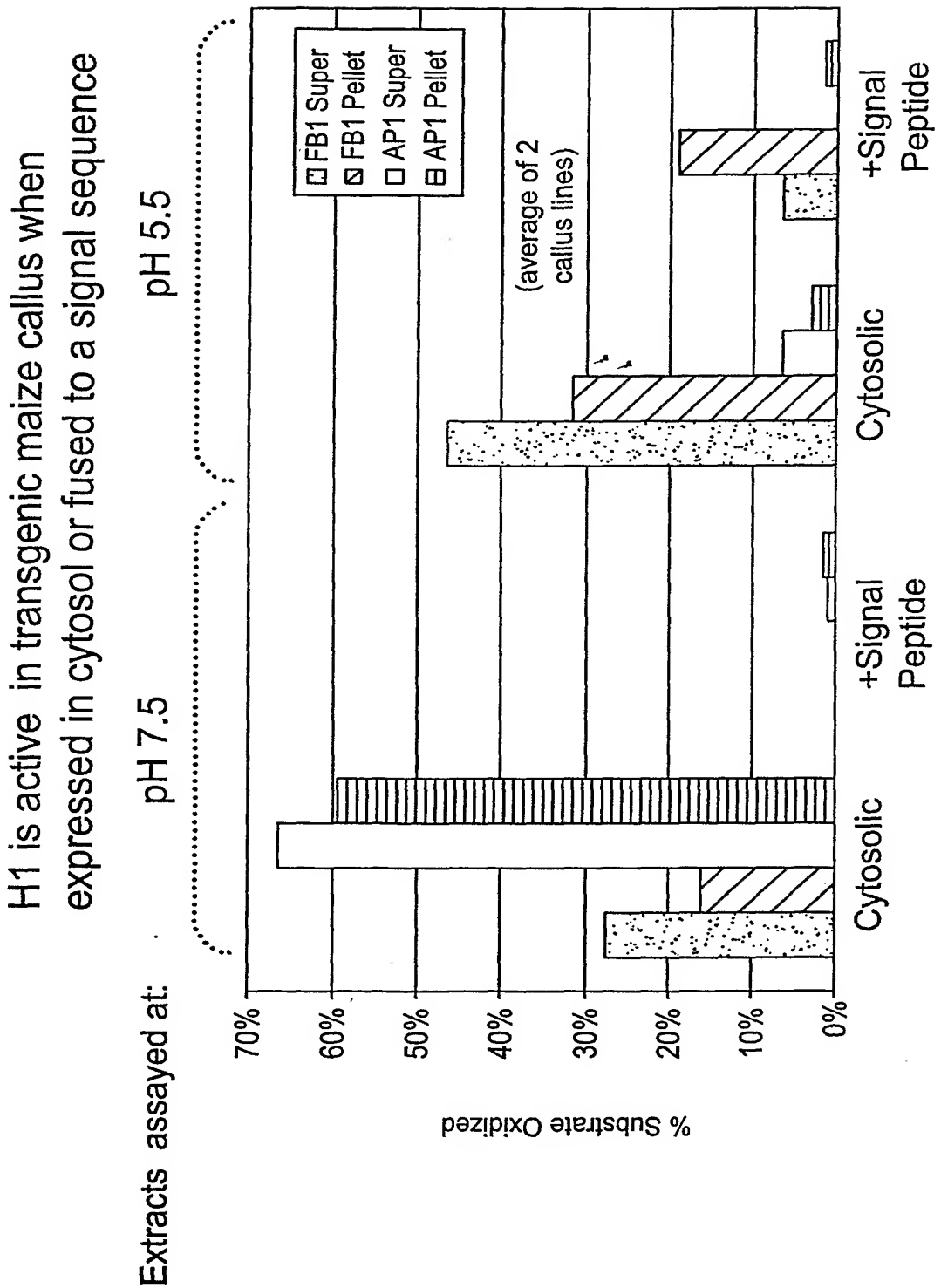

H1 is active in transgenic maize callus when expressed in cytosol or fused to a signal sequence
Extracts assayed at:
pH 7.5
pH 5.5
FB1 Super
FB1 Pellet
AP1 Super
AP1 Pellet
(average of 2 callus lines)
% Substrate Oxidized
70%
60%
50%
40%
30%
20%
10%
0%
Cytosolic
+Signal Peptide
Cytosolic
+Signal Peptide
Wild type APAO
H1- APAO

Fig. 7

Fig. 8A

	rfu/min/ug (prior to preincubation)	%activity (after preincubation)
h1	1325	0.21
g6	1804	0.59
1b6	1612	0.55
1h8	1804	0.35
3e7	1558	0.71

Fig. 8B

	km	kcat
h1	259.12	2164.50
g6	154.19	1612.90

Fig. 8C

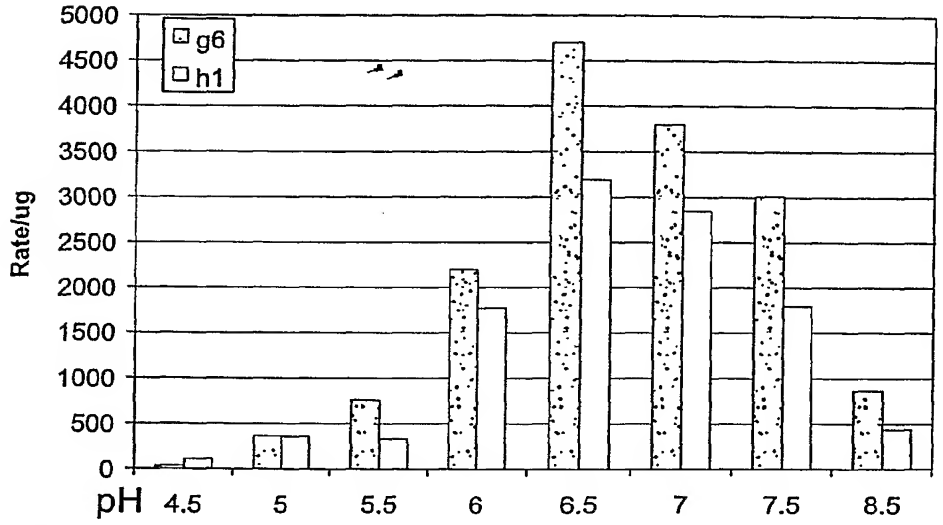
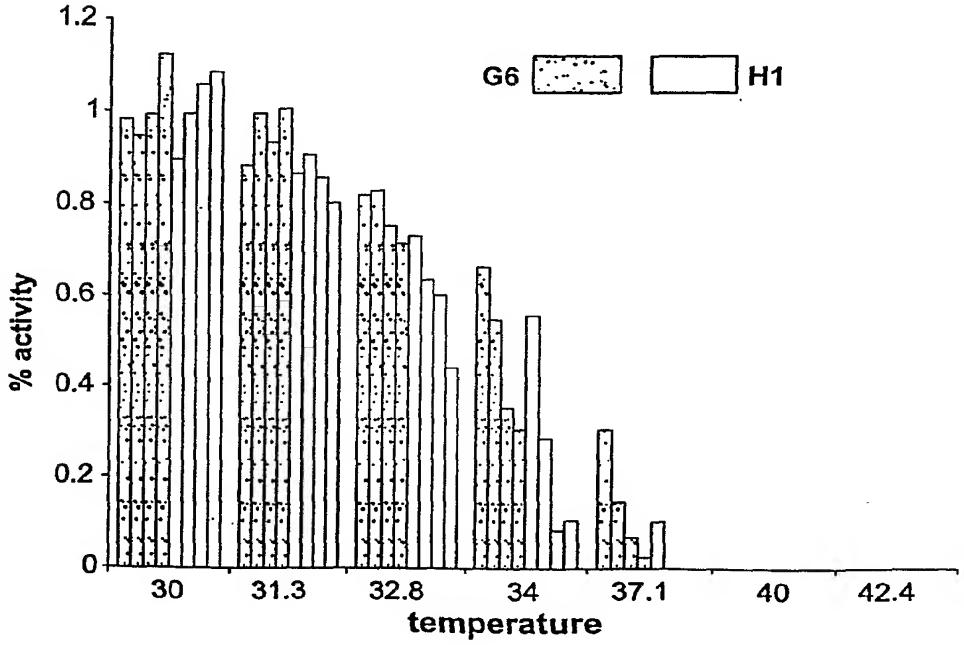
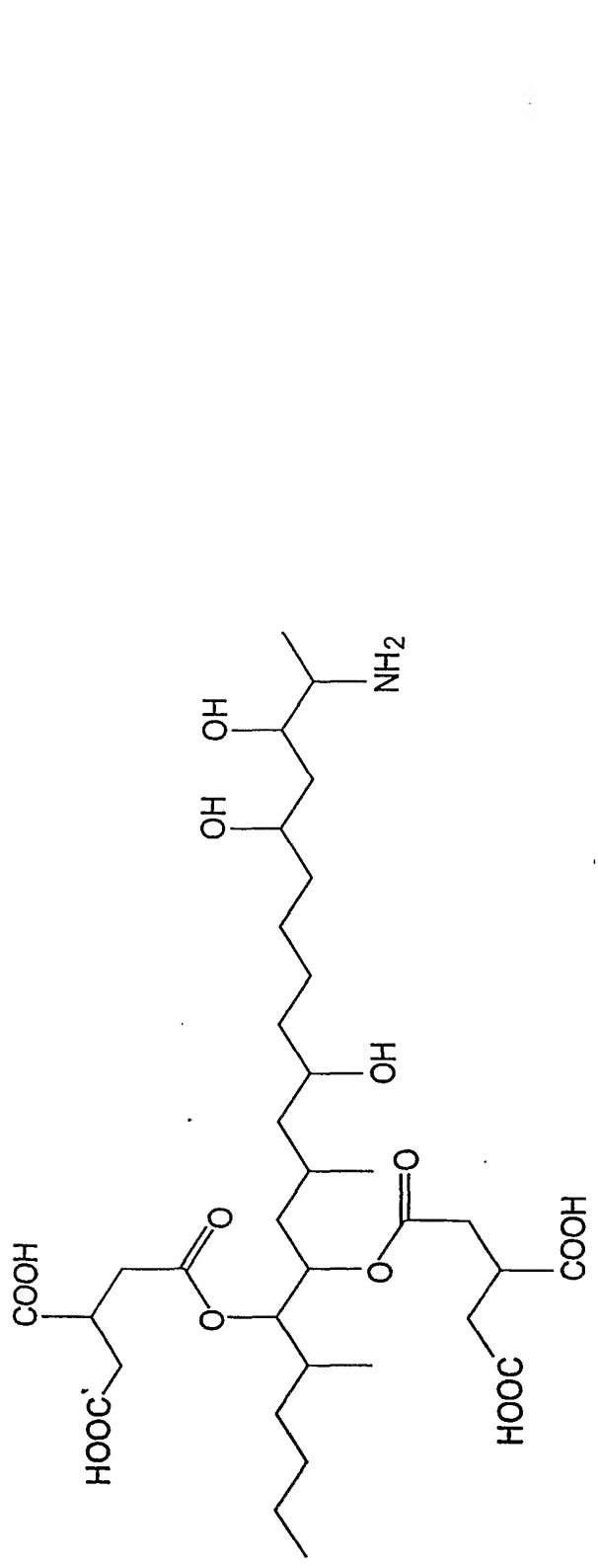
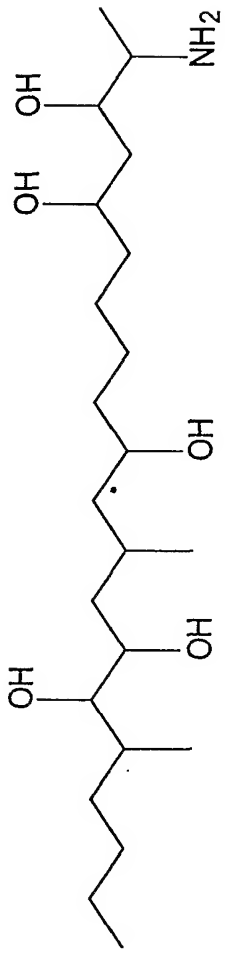


Fig. 8D





FB1



AP1

Fig. 9

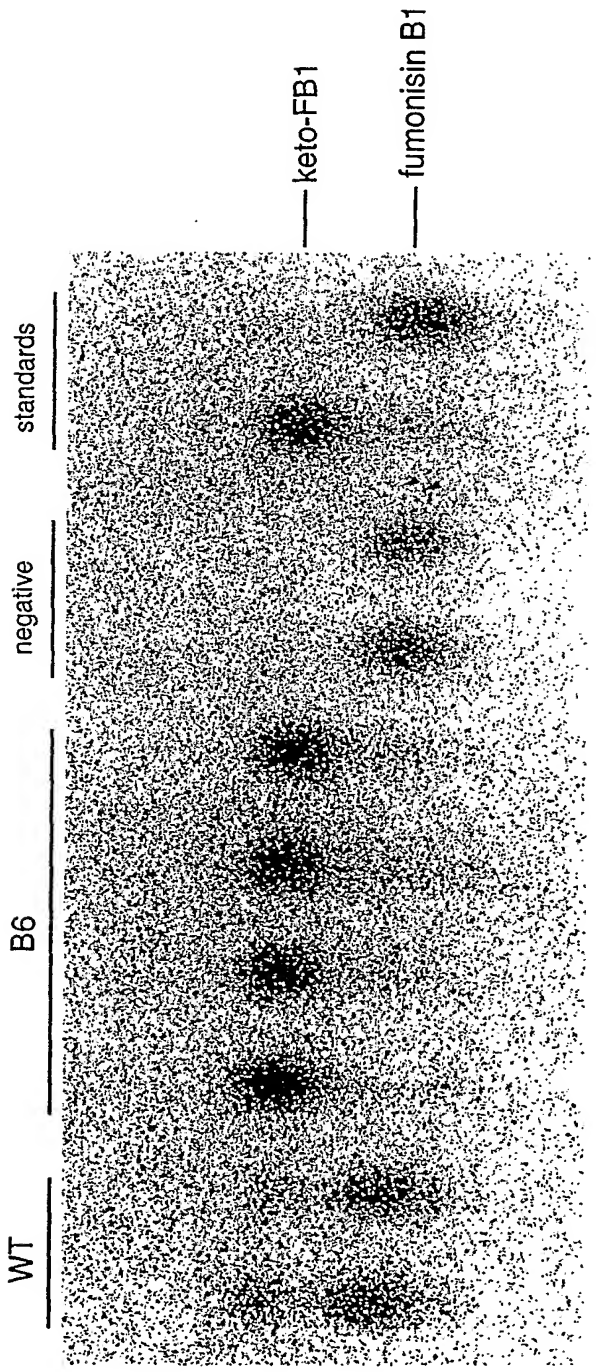


Fig. 10

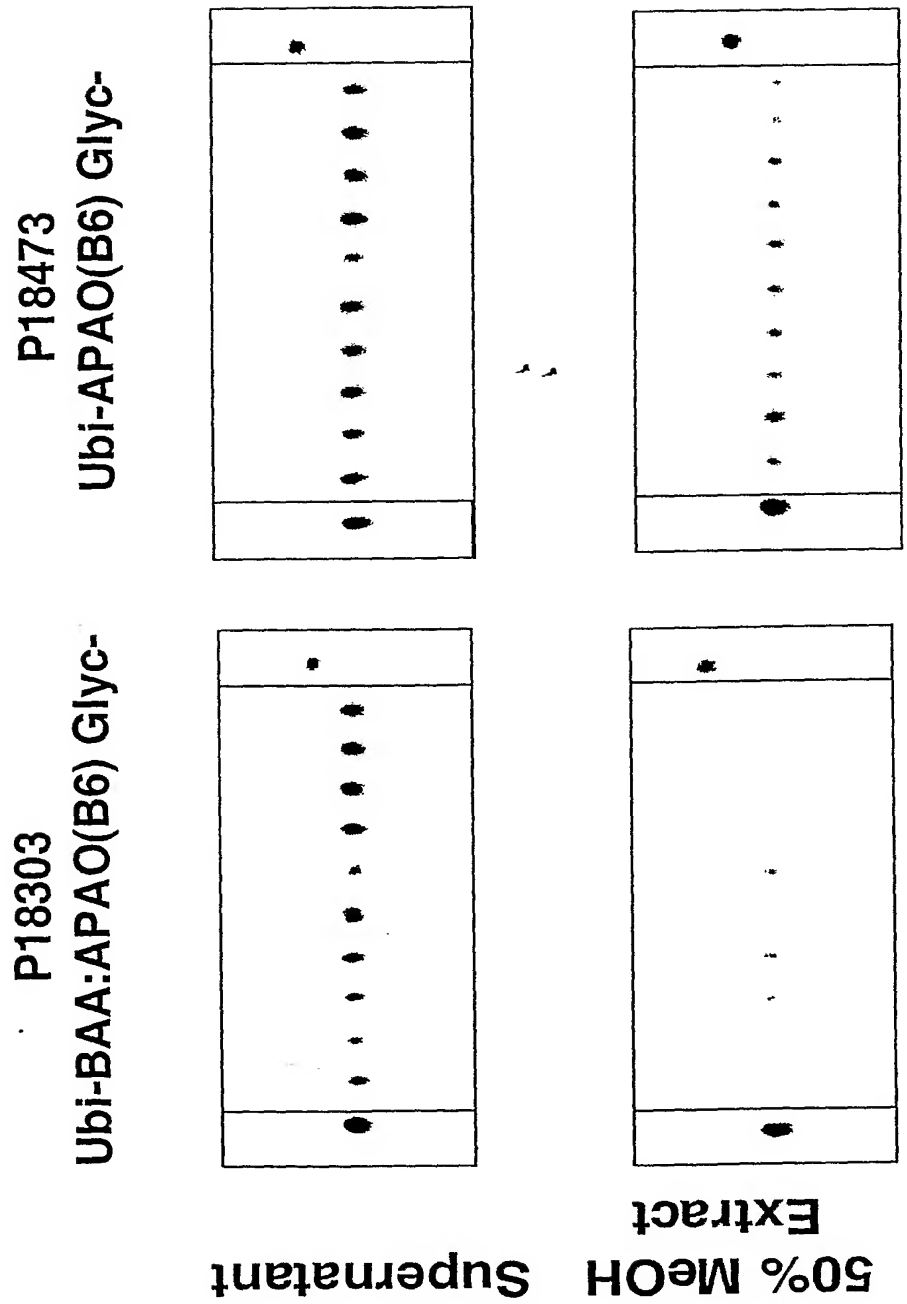

P18473
Ubi-APAO(B6) Glyc-
P18303
Ubi-BAA:APAO(B6) Glyc-
Supernatant
50% MeOH
Extract

Fig. 11

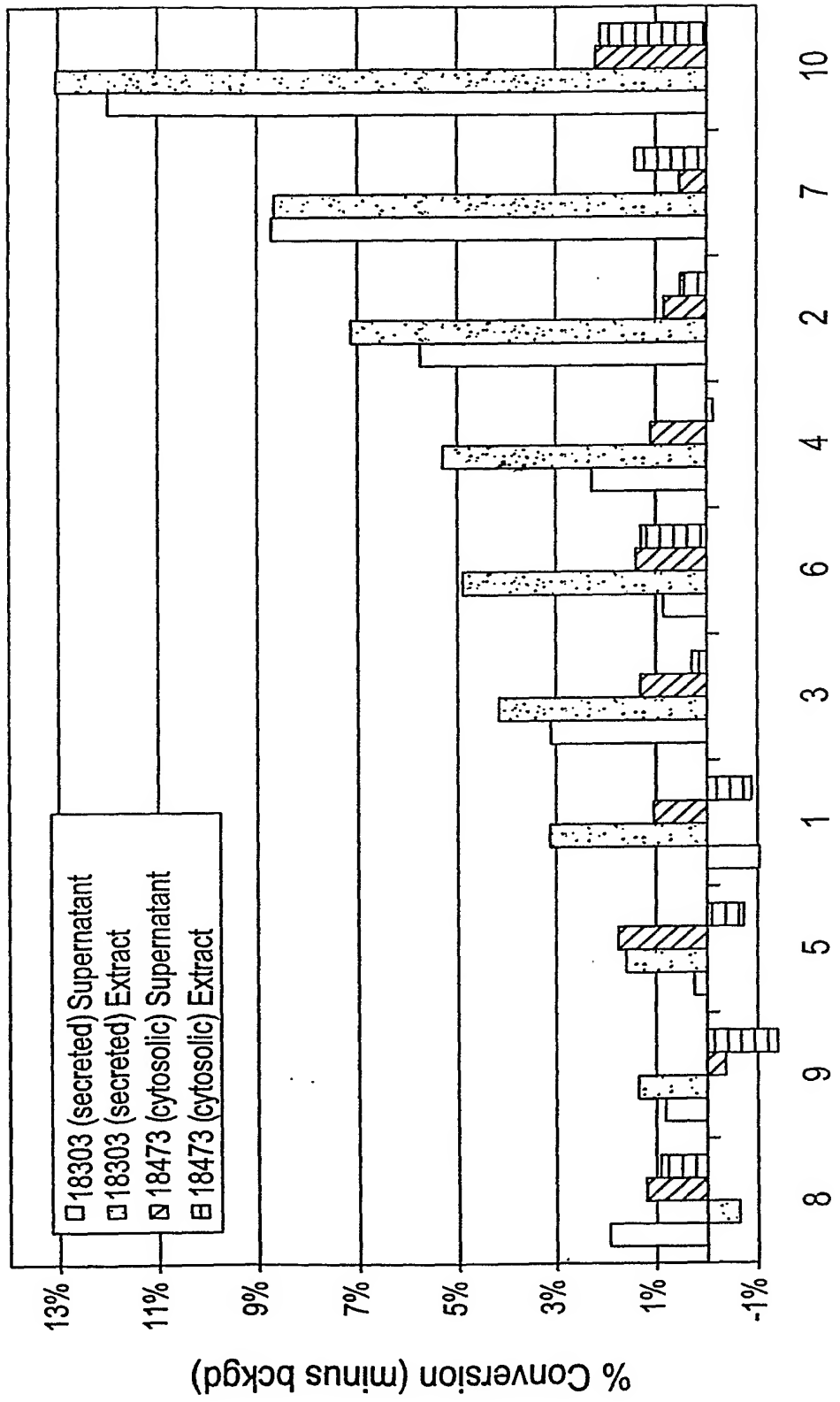

18303 (secreted) Supernatant
18303 (secreted) Extract
18473 (cytosolic) Supernatant
18473 (cytosolic) Extract
13%
11%
9%
7%
5%
3%
1%
-1%
% Conversion (minus bckgd)
1
2
3
4
5
6
7
8
9
10
Embryo Number

Fig. 12

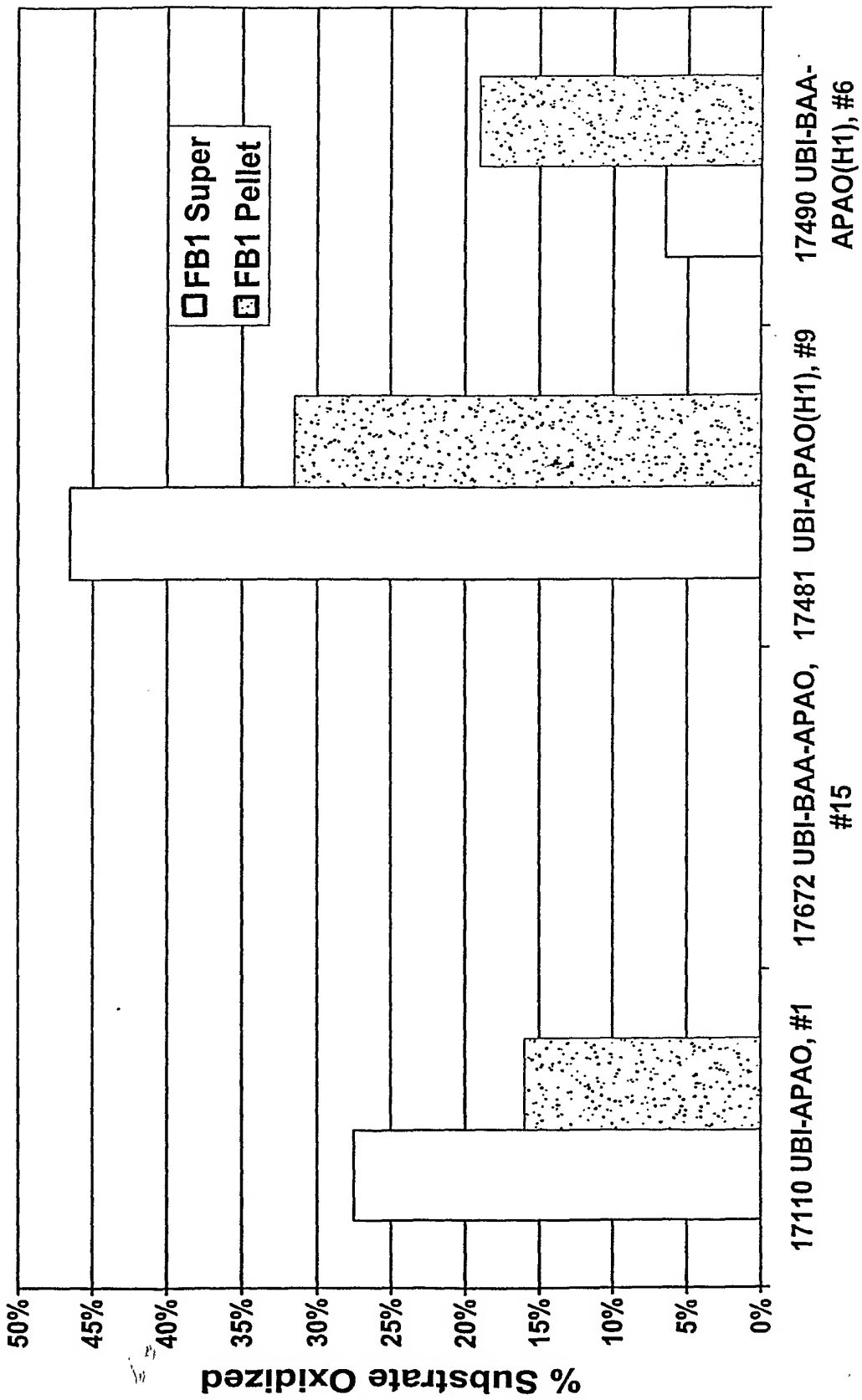

% Substrate Oxidized
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
FB1 Super
FB1 Pellet
17110 UBI-APAO, #1
17672 UBI-BAA-APAO, #15
17481 UBI-APAO(H1), #9
17490 UBI-BAA-APAO(H1), #6
Construct/Callus Line

Fig. 13